RESEARCH ARTICLE

Altitude ammonia-oxidizing bacteria and archaea in soils of Mount Everest

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Abstract

To determine the abundance and distribution of bacterial and archaeal ammonia oxidizers in alpine and permafrost soils, 12 soils at altitudes of 4000–6550 m above sea level (m a.s.l.) were collected from the northern slope of the Mount Everest (Tibetan Plateau), where the permanent snow line is at 5800–6000 m a.s.l. Communities were characterized by real-time PCR and clone sequencing by targeting on *amo*A genes, which putatively encode ammonia monooxygenase subunit A. Archaeal *amo*A abundance was greater than bacterial *amo*A abundance in lower altitude soils ($\leq$ 5400 m a.s.l.), but this situation was reversed in higher altitude soils ($\geq$5700 m a.s.l.). Both archaeal and bacterial *amo*A abundance decreased abruptly in higher altitude soils. Communities shifted from a *Nitrosospira amo*A cluster 3a-dominated ammonia-oxidizing bacteria community in lower altitude soils to communities dominated by a newly designated *Nitrosospira* ME and cluster 2-related groups and *Nitrosomonas* cluster 6 in higher altitude soils. All archaeal *amo*A sequences fell within soil and sediment clusters, and the proportions of the major archaeal *amo*A clusters changed between the lower altitude and the higher altitude soils. These findings imply that the shift in the relative abundance and community structure of archaeal and bacterial ammonia oxidizers may result from selection of organisms adapted to altitude-dependent environmental factors in elevated soils.

Introduction

Until recently, chemolithoautotrophic ammonia-oxidizing bacteria (AOB) within the *Proteobacteria* were thought to be the major microorganisms performing ammonia oxidation, the first and rate-limiting step of nitrification. Metagenomic studies first suggested that ammonia oxidation was potentially driven by members within the domain archaea that contained putative ammonia monooxygenase subunits (*amo*A, *amo*B and *amo*C) (Venter *et al.*, 2004; Treusch *et al.*, 2005). The first successful isolation of a mesophilic crenarchaeon, *Nitrosopumilus maritimus*, growing autotrophically with ammonia as the sole energy source, confirmed the association of these archaeal ammonia monooxygenase genes with ammonia oxidation (Könneke *et al.*, 2005). Given the functional significance and conserved phylogeny, the *amo*A gene was used as a molecular marker for both AOB and these putative ammonia-oxidizing archaea (AOA). Subsequent studies have demonstrated that archaeal *amo*A genes are ubiquitous in marine environments (Francis *et al.*, 2005; Wuchter *et al.*, 2006), wastewater bioreactors (Park *et al.*, 2006), terrestrial hot springs (Weidler *et al.*, 2007; Reigstad *et al.*, 2008) and soils (He *et al.*, 2007; Nicol *et al.*, 2008; Shen *et al.*, 2008). Active expression of archaeal *amo*A gene has also been detected in natural environments (Lam *et al.*, 2007; Tourna *et al.*, 2008) and enrichment cultures (Hatzenpichler *et al.*, 2008).

Archaeal *amo*A genes are more abundant than those of bacteria in various environments. In marine environments, archaeal *amo*A gene abundance exceeded that of bacteria by up to four orders of magnitude (Wuchter *et al.*, 2006; Lam *et al.*, 2007; Mincer *et al.*, 2007). In a study of 12 pristine and agricultural soils crossing three climate zones, archaeal *amo*A gene was up to 3000 times that of bacterial *amo*A

and correlated with *Crenarchaeota*-specific lipids (Leininger *et al.*, 2006). Consistently higher abundance of archaeal *amo*A genes has also been reported in Chinese acid and alkaline soils with different fertilization regimes (He *et al.*, 2007; Shen *et al.*, 2008) and in Scottish agricultural plots (Nicol *et al.*, 2008). The numerical dominance of archaeal over bacterial *amo*A genes in natural environments suggested its putative greater ecological function in global nitrogen cycling, but the presence or the high abundance of a functional gene does not mean that the function is operating (Prosser & Nicol, 2008). Moreover, some recent studies reported greater abundance of AOB *amo*A genes in estuarine sites (Caffrey *et al.*, 2007), in coastal sediments with increasing salinity (Mosier & Francis, 2008; Santoro *et al.*, 2008) and in anoxic sediments (Jiang *et al.*, 2009). It is important to understand whether there are more habitats in which AOB dominate, especially in soils, and the physical and chemical factors that determine the relative abundance and diversity of these two distinct groups of ammonia oxidizers.

A number of studies have shown that the distribution, community composition and abundance of AOB and AOA are influenced considerably by temperature (Avrahami *et al.*, 2003), pH (Nicol *et al.*, 2008), simulated global changes (Horz *et al.*, 2004), salinity (Mosier & Francis, 2008; Santoro *et al.*, 2008) and fertilization regime (He *et al.*, 2007; Shen *et al.*, 2008). Distinct changes in the AOA community composition have also been observed in a soil profile, in which AOB were not detected (Hansel *et al.*, 2008), and a marked decrease in the abundance of archaeal *amo*A gene was found with increasing depth, from subsurface waters to 4000 m depth in the North Atlantic Ocean (Agogue *et al.*, 2008). However, AOB and AOA communities in high-altitude soils have not been investigated.

The Tibetan Plateau is the Earth's largest (2×10^6 km^2) and highest [mean altitude 4500 m above sea level (m a.s.l.)] plateau. Tectonic uplifting of the Tibetan Plateau has led to a unique system, with snow cover, high UV exposure and lower oxygen and nutrient concentrations. As a result, microbial communities in topsoil layers encounter extreme conditions that may lead to unique survival adaptations and differences in the community composition. The Tibetan Plateau is also one of the most special regions sensitive to global climate change (Wu *et al.*, 2007) and the global warming and elevated CO_2 concentration may have a complex impact on below-ground microorganisms (Xu *et al.*, 2009). In the present study, real-time PCR and clone-library sequencing were used to characterize the distribution and community composition of AOA and AOB in these alpine soils, where the altitude-dependent environmental factors and changing global climate may select for particular populations of ammonia oxidizers.

Materials and methods

Sampling sites and soil collection

During the fourth expedition on Mount Everest of the Chinese Academy of Sciences from April to June 2005, 12 soil samples (M1–M12) were collected from the northern slope of Mount Everest and adjoining areas (Table 1). M1–M3 are field or farmland soils in the Rikaze area, Tibet, at an altitude of 4000 m a.s.l. M4–M12 are bare soils with no visible plant development (Fig. 1). The permanent snow line is at 5800–6000 m a.s.l. For each site, three subsamples were removed from the soil surface (0–10 cm depth) and were mixed after removal of large stones and snow. Samples were transported to a refrigerator with a temperature of –10 °C in 2 days after collecting and stored until further analysis. Soils were passed through a 2.0-mm sieve before use.

Soil chemical analysis

Soil pH was determined with a soil to water ratio of 1 : 10. Soil organic matter was determined using the $K_2Cr_2O_7$ oxidation method, and total nitrogen was determined using the Kjeldahl method (Bremner, 1996).

DNA extraction

DNA was extracted from 0.8 to 1.0 g (fresh weight) of soil using the FastDNA SPIN Kit for Soil (Q BIOgene Inc.,

Table 1. Soil characteristics for samples along the Mount Everest on the Tibetan Plateau

Soil code	Altitude (m)	Location	pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})
M1	4000	Lazi field soil	8.6	8.4	0.51
M2	4000	Rikaze farmland soil	8.7	10.8	0.66
M3	4000	Dazhuka field soil	8.6	12.6	0.49
M4	5350	N28 08.385 E86 51.533	9.1	4.1	0.14
M5	5400	N28 08.336 E86 51.602	9.0	6.3	0.21
M6	5700	N28 05.376 E86 54.469	9.1	4.3	0.06
M7	5850	N28 04.882 E86 55.256	9.0	4.2	0.07
M8	6000	N28 03.892 E86 55.605	9.0	2.9	0.06
M9	6150	N28 03.030 E86 56.294	9.1	3.7	0.09
M10	6300	N28 02.341 E86 56.686	9.0	4.4	0.11
M11	6450	N28 01.773 E86 56.337	9.0	4.4	0.11
M12	6550	N28 01.570 E86 55.987	9.0	5.9	0.09

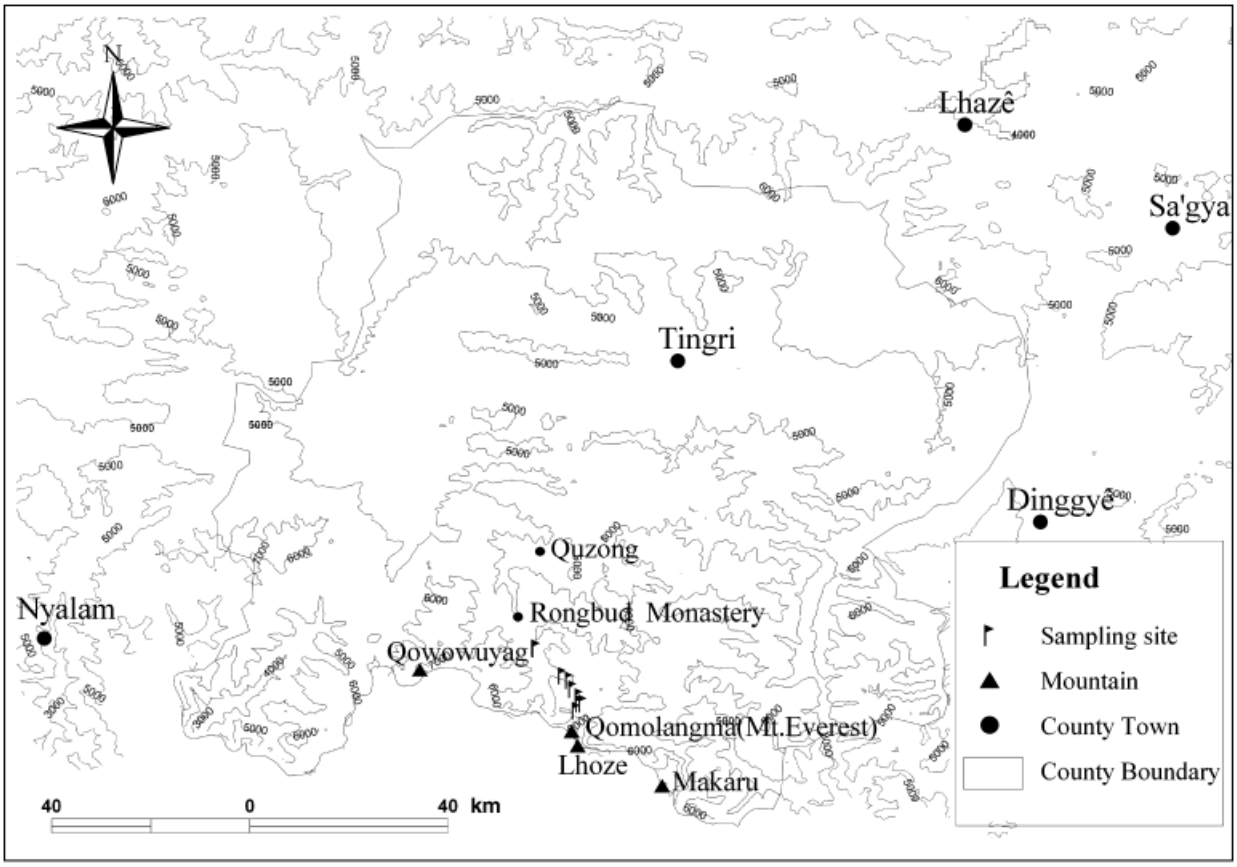

Fig. 1. Location of soils sampled in the Mount Everest, on the Tibetan Plateau.

Carlsbad, CA) with a bead beating time of 20 s and a speed setting of 5.5 m. Extracted DNA was checked on a 1% agarose gel and the concentration was determined using a Nanodrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE).

Quantification of *amo*A genes by real-time PCR

Primer pairs amoA1F/amoA2R (Rotthauwe *et al.*, 1997) and Arch-amoAF/Arch-amoAR (Francis *et al.*, 2005) were used for real-time PCR quantification of bacterial and archaeal *amo*A genes, respectively. Real-time PCR was performed on an iCycler iQ 5 thermocycler (Bio-Rad). The 25 μL reaction volume contained 12.5 μL SYBR® Premix Ex Taq™ (TaKaRa Bio Inc., Shiga, Japan), 0.4 mg mL^{-1} bovine serum albumin, 400 nM of each AOB primer or 200 nM each of AOA primer and 2 μL of 10-fold diluted or undiluted extracted DNA (1–10 ng) as a template. Three replicates were analyzed for each sample. Amplifications were carried out as follows: 95 °C for 1 min, followed by 40 cycles of 10 s at 95 °C, 30 s at 55 °C for AOB or 53 °C for AOA, 1 min at 72 °C and plate read at 83 °C. Melting curve analysis was performed at the end of PCR runs to check the specificity of the products. PCR products amplified from extracted DNA with the primers for real-time PCR assays were gel-purified and ligated into the pGEM-T Easy Vector (Promega, Madison), and the resulting ligation products were transformed into *Escherichia coli* JM109 competent cells following the manufacturer's instructions. After reamplification with the vector-specific primers T7 and SP6, the positive clones were selected to extract plasmid DNA using a MiniBEST Plasmid Purification Kit (TaKaRa Bio Inc.) and used as *amo*A gene standards. The plasmid DNA concentration was determined on a Nanodrop® ND-1000 UV-Vis Spectrophotometer (NanoDrop Technologies), and *amo*A gene copy number was calculated directly from the concentration of extracted plasmid DNA. Tenfold serial dilutions of a known copy number of the plasmid DNA were subjected to real-time PCR in triplicate to generate an external standard curve. PCR efficiency and correlation coefficients for standard curves were 95.4% and $r^2 = 0.996$ for AOB and 93.4% and $r^2 = 0.996$ for AOA.

Cloning and sequence analysis of bacterial and archaeal *amo*A genes

PCR products of bacterial and archaeal *amo*A genes amplified with the primers for the real-time PCR assays were

purified and cloned, as described above, for each site, generating 12 clone libraries for bacterial and 12 for archaeal *amo*A genes. Clones were screened for inserts of the expected size with the vector-specific primers T7 and SP6. For restriction fragment length polymorphism (RFLP) assay, approximately 60 positive clones from each clone library were digested with restriction endonuclease MboI (Takara Bio Inc.). Digested DNA fragments were separated by electrophoresis on a 2% agarose gel and imaged using a GBOX-HR Gel Documentation System (Syngene, UK) after ethidium bromide staining.

The RFLP patterns were grouped and one to three clones representing unique band types were sequenced. Sequences were subjected to homology analysis using the software DNAMAN version 6.0.3.48 (Lynnon Biosoft). Sequences of chimeric origin were checked by partial treeing analysis and compared with GenBank sequences using BLASTN searches. For each clone library, for sequence types that exhibited $>$ 98% identity to each other, only one representative was used for construction of the tree. The GenBank sequences most similar to clone sequences in this study and reference sequences for defining clusters were included in phylogenetic tree construction. Phylogenetic analysis based on nucleotide sequences was performed using MEGA version 4.0 (Tamura *et al.*, 2007) and a neighbor-joining tree was constructed using Kimura two-parameter distance with 1000 replicates to produce bootstrap values (1000 replicates).

The sequences determined in this study were deposited in the GenBank database and assigned accession numbers from FJ853214 to FJ853366.

Statistical analyses

*amo*A gene abundance data were log-transformed to provide variance homogeneity. Statistical analyses were performed using SPSS version 13.0. Paired-samples *t*-tests were used to compare archaeal and bacterial *amo*A gene copy numbers and independent-sample *t*-tests were used to compare archaeal or bacterial *amo*A gene copy numbers at lower altitudes and at higher altitudes. Bivariate correlations were carried out to link different parameters. $P < 0.05$ was considered to be significant.

Results

Soil properties

Three soils (M1–M3) at an altitude of 4000 m a.s.l. were sampled from field or farmland with pH values of 8.6–8.7. Organic matter in these three soils varied between 8.4 and 12.6 g kg^{-1} soil and total nitrogen varied between 0.51 and 0.66 g kg^{-1} soil (Table 1). Soils (M4–M12) at altitudes above 5000 m a.s.l. were less well developed with a high content of gravel, and pH was in the range 9.0–9.1. The organic matter content in these soils ranged from 2.9 to 6.3 g kg^{-1} soil, and total nitrogen from 0.06 to 0.21 g kg^{-1} soil. These values are much lower than in M1–M3 soils and showed no significantly negative correlation with altitude (Table 2).

AOA and AOB abundance

The abundance of archaeal and bacterial *amo*A genes decreased abruptly in soils above 5400 m a.s.l. and significant differences in archaeal and bacterial *amo*A abundances were observed between lower altitude (M1–M5, $\leq$ 5400 m a.s.l.) and higher altitude (M6–M12, $\geq$ 5700 m a.s.l.) soils (d.f. = 10, $P < 0.05$; Fig. 2a). Differences were also seen in the relative abundances of archaeal and bacterial *amo*A genes. Archaeal *amo*A abundance in lower altitude soils (5.17×10^7–3.79×10^8 g^{-1} soil) was significantly higher than bacterial *amo*A abundance (6.97×10^5–1.59×10^7 g^{-1} soil) (d.f. = 4, $P < 0.001$; Fig. 2a). In contrast, at high altitude, archaeal *amo*A abundance (4.63×10^3–2.72×10^4 g^{-1} soil) was less than that for bacteria (5.41×10^4–3.1×10^6 g^{-1} soil) (d.f. = 6, $P < 0.001$; Fig. 2a). As a consequence, the mean $\log_{10}$ ratio of archaeal : bacterial *amo*A decreased from 1.45 in low-altitude soils to -1.34 in high-altitude soils (Fig. 2b), with significantly negative and positive correlations, respectively, with altitude and archaeal *amo*A abundance (Table 2). There is a

Table 2. Correlation analyses among the abundance of AOA/AOB, altitude, pH, organic matter and total nitrogen content

	Kendall's correlation coefficient					
	Abundance of AOA	Abundance of AOB	Log ratio of AOA to AOB	Altitude	pH	Organic matter
Abundance of AOB	0.667**					
Log ratio of AOA to AOB	0.606**	0.273				
Altitude	−0.543**	−0.419	−0.605**			
pH	−0.377	−0.377	−0.233	0.312		
Organic matter	0.606**	0.515**	0.394	−0.326	0.664**	
Total nitrogen content	0.788**	0.697**	0.394	−0.388	0.521*	0.576**

*Correlation is significant at the 0.05 level (two-tailed).
**Correlation is significant at the 0.01 level (two-tailed).

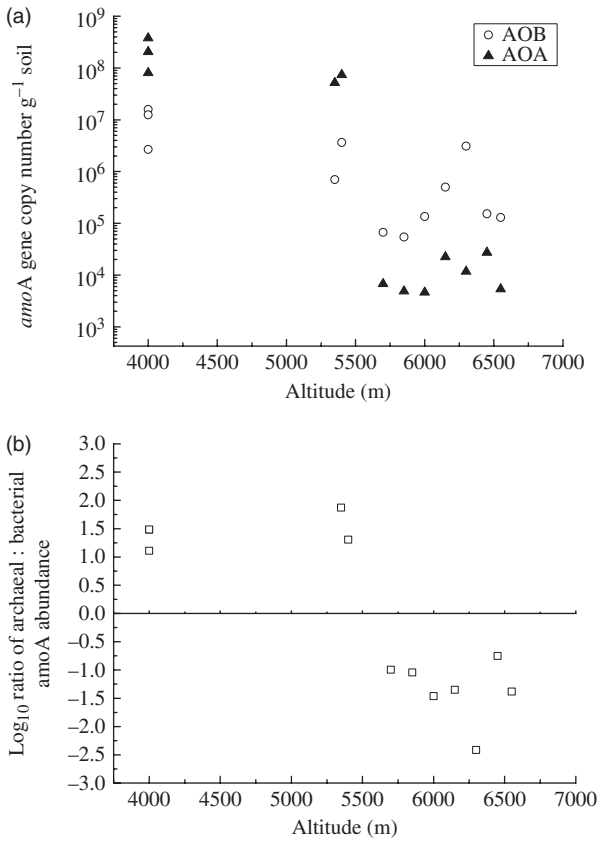

Fig. 2. Abundance of the bacterial and archaeal *amo*A along the altitude gradient of Mount Everest on the Tibetan Plateau. (a) The abundance of archaeal and bacterial *amo*A; (b) $\log_{10}$ ratio of archaeal : bacterial *amo*A abundance.

significantly negative correlation between the abundance of AOA and altitude, and a nonsignificant negative correlation between the abundance of AOB and altitude (Table 2). The abundance of AOA was positively correlated with the abundance of AOB, and significantly positive correlations were also observed between both AOA and AOB abundance and organic matter and total nitrogen (Table 2).

Community composition of AOA and AOB

Phylogenetic trees of the bacterial and archaeal *amo*A gene sequences and related NCBI sequences are shown in Figs 3 and 4. All AOB sequences fell within *Nitrosospira amo*A clusters 3a.1, 3a.2, ME, cluster 2-related and *Nitrosomonas amo*A clusters 6 and 7 (Avrahami *et al.*, 2002; Avrhami & Conrad, 2003). Sequences in cluster 3a fell within two subclusters 3a.1 and 3a.2 with bootstrap value at 90, and two distinct clades were designated as cluster ME and cluster 2-related. Cluster ME was related to, but outside of, cluster 1 and 4 groups with a bootstrap value of 90 and cluster 2-related sequences fell outside of cluster 2.

The proportion of sequence types representing various clusters in each clone library was calculated based on RFLP analysis of clones. In lower altitude soils (M1–M5), AOB communities were dominated by representatives of clusters 3a.1 and 3a.2, constituting 91.8–100% in each clone library, and also contained representatives of cluster 6 (Fig. 5a). The proportions of cluster 3a.1 and 3a.2 decreased in higher altitude soils (M6–M12) and only cluster 3a.1 was detected in two of seven high-altitude soils, in a low proportion (2.5–18.8%), while the cluster ME, cluster 2-related and cluster 6 sequences appeared in high proportions in these soils (Fig. 5a). Sequences falling within cluster ME and cluster 2-related groups were exclusively from the higher altitude soils (M6–M12) and accounted for 30.3–81.3% of the total clones in each clone library. Cluster ME sequences had $< 94\%$ similarity to the *amo*A-like sequences in GenBank database, with the exception of several clones, including AOB_M10_20, AOB_M11_29, AOB_M6_18 and AOB_M12_10, which shared 98% similarity to the unclassified sequence AY667607 from plant rhizosphere soil (Hawkes *et al.*, 2005). Most sequences affiliated with the cluster 2-related group shared $< 94\%$ similarity to sequences in GenBank database. The proportion of cluster 6 sequences was greater in higher altitude soils. Cluster 7 sequences were detected only in some higher altitude soils. In summary, therefore, the data indicate substantial differences in bacterial ammonia oxidizer community composition between the lower and the higher altitude soils.

All AOA sequences, with the exception of AOA_M11_1, fell within soil/sediment lineage that was dominated by sequences from soil and sediment environments and was grouped into three distinct clades (Fig. 4). Clades 1 and 2 formed a monophyletic lineage with modest bootstrap support, and clade 3 was related to, but outside this monophyletic clade, which showed a topology similar to that observed by Hansel *et al.* (2008) for sequences from a continuous soil profile (Fig. 4). These were designated as soil and sediment clusters 1, 2 and 3 (Fig. 4). Soil and sediment clusters 1 and 2 represented the majority of all AOA clones, were present in all soils at varying proportions and corresponded to Saturated C Horizon and partial Mixed B and C Horizon clusters of Hansel *et al.* (2008), respectively (Fig. 5b). In lower altitude soils, more soil and sediment cluster 1 sequences (70–91.2%) and less cluster 2 sequences (5.8–22.5%) were obtained, while more cluster 2 sequences appeared with proportions of 31.8–92.3% in higher altitude soils (Fig. 5b). These sequences are most closely related to *amo*A sequences retrieved from various soils (Leininger *et al.*, 2006; Chen *et al.*, 2008; Hansel *et al.*, 2008; Shen *et al.*, 2008), alpine soils (G.W. Nicol & J.I. Prosser, unpublished data), sediments (Francis *et al.*, 2005; Jiang *et al.*, 2009), soil fosmid 54d9 (Treusch *et al.*, 2005) and newly cultivated strain *Candidatus* Nitrososphaera gargensis

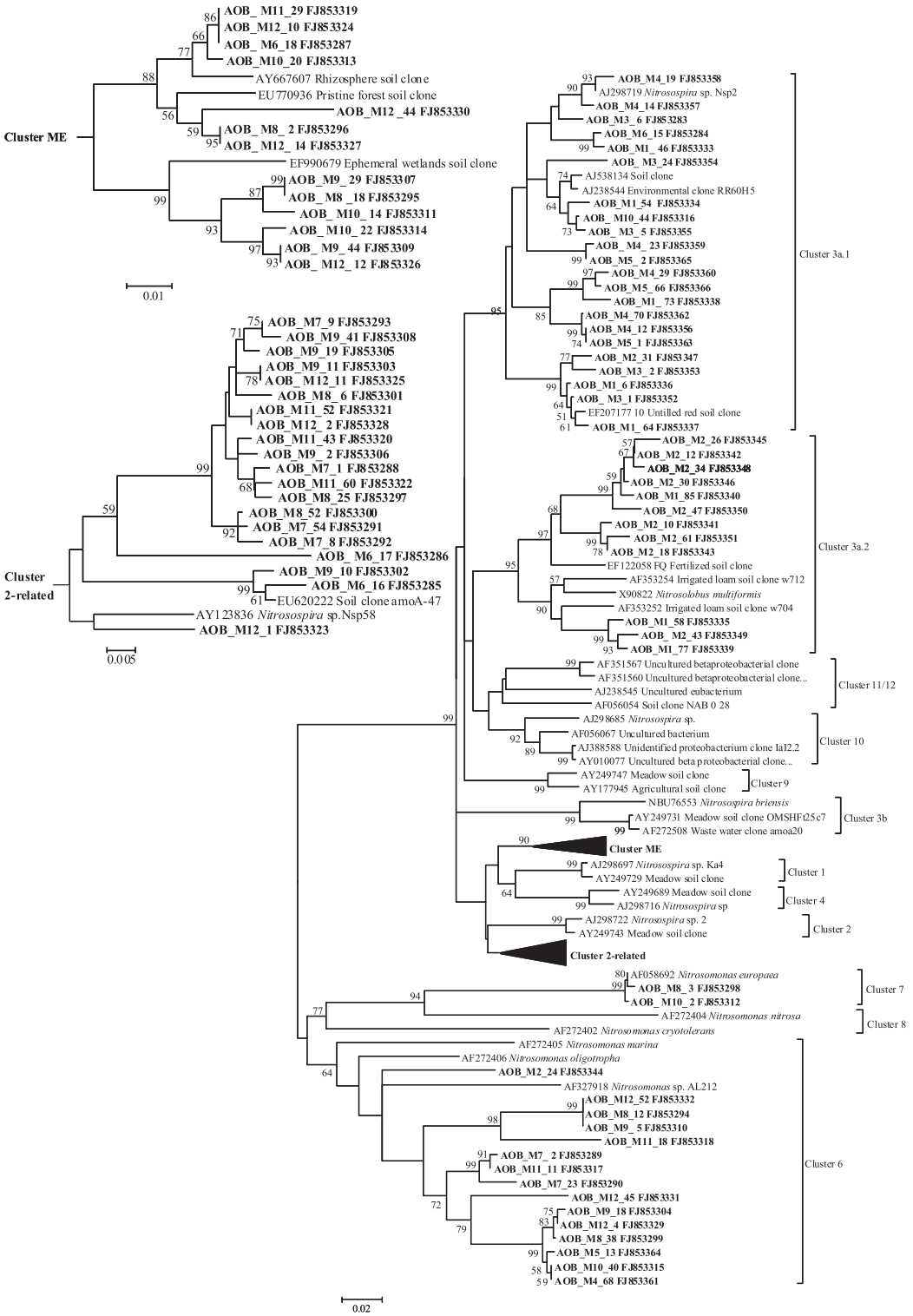

Fig. 3. Neighbor-joining phylogenetic tree of bacterial *amo*A sequences (465-bp fragment) retrieved from Mount Everest, on the Tibetan Plateau. Clones from this study are shown in bold with the name AOB, followed by soil code (M1–M12), and then by clone code. The accession number follows each clone. Bootstrap values (> 50) are indicated at branch points.

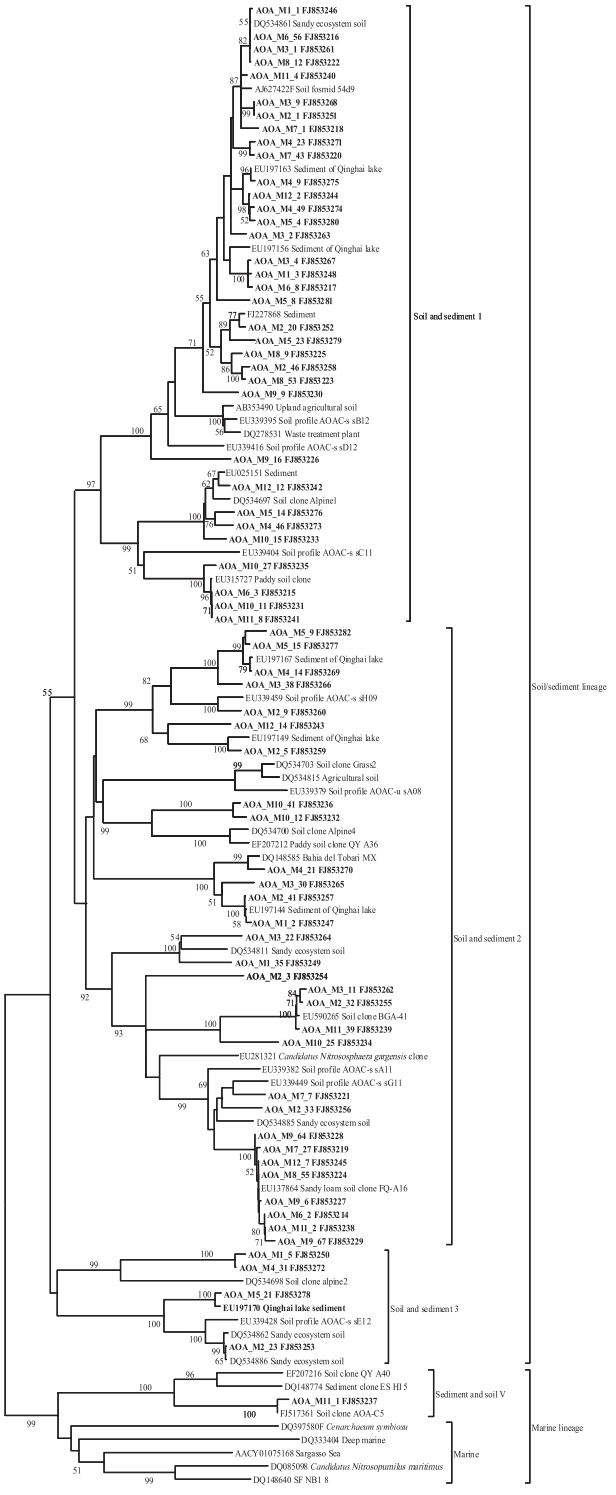

Fig. 4. Neighbor-joining phylogenetic tree of archaeal *amo*A sequences (594-bp fragment) retrieved from Mount Everest, on the Tibetan Plateau. Clones from this study are shown in bold with the name AOA, followed by soil code (M1–M12), and then by clone code. The accession number follows each clone. Bootstrap values (> 50) are indicated at branch points.

(Hatzenpichler *et al.*, 2008). Sequences falling within soil and sediment cluster 3 were present in four of five lower altitude soils with proportions of 1.8–22%.

The sequence AOA_M11_1, representing 24% of soil clones at an altitude of 6450 m a.s.l., fell within sediment and soil V cluster in marine lineage, which was previously characterized as being dominated by marine-originating sequences, but was grouped into two distinct clades (sediment and soil V cluster and marine cluster). The sediment and soil V cluster, defined by Nicol *et al.* (2008), was distinct from the marine cluster and mainly from soil and sediment environments (Fig. 4).

Discussion

Archaeal *amo*A abundance was markedly higher than bacterial *amo*A abundance in lower altitude soils (M1–M5). The result was consistent with many studies showing greater abundance of archaeal than bacterial *amo*A genes in most ecosystems (Leininger *et al.*, 2006; Wuchter *et al.*, 2006; He *et al.*, 2007; Lam *et al.*, 2007; Mincer *et al.*, 2007; Nicol *et al.*, 2008; Shen *et al.*, 2008; Di *et al.*, 2009). Intriguingly, archaeal *amo*A abundance decreased by up to four orders of magnitude between lower altitude and higher altitude soils, while bacterial *amo*A abundance decreased only 10-fold. As a result, the relative abundance of AOA and AOB shifted drastically, with AOB outnumbering AOA by 52 times on average in higher altitude soils (M6–M12). Variation in this ratio has also been described for coastal aquifer sediments, with greater abundance of AOA over AOB at low, but not high salinity (Mosier & Francis, 2008; Santoro *et al.*, 2008), and in oxic lake water, but not in anoxic sediment (Jiang *et al.*, 2009). Caffrey *et al.* (2007) also found that AOB were more abundant than AOA in several estuarine sites. The $\log_{10}$ ratio of AOA to AOB correlated negatively with altitude and positively with AOA abundance, and AOA abundance was negatively related to altitude, indicating that AOA may be more sensitive than AOB in response to elevated soil conditions. Both AOA abundance and AOB abundance were positively correlated to organic matter and total nitrogen, suggesting that these may also determine the distribution of AOA and AOB in alpine soils, by providing the nutrient and substrate for microbial mineralization. It was also found that warming at high-elevation sites was more pronounced than at low elevations (Giorgi *et al.*, 1997), and thus might exert different impacts on soil microbial populations for the low and high altitudes.

The abundance of AOA and AOB decreased considerably in soil at an altitude of 5700 m a.s.l. and then maintained a lower level in higher altitude soils. A corresponding shift was observed in AOB community composition between the lower altitude and the higher altitude soils. In lower altitude soils, the AOB community was dominated by *Nitrosospira*

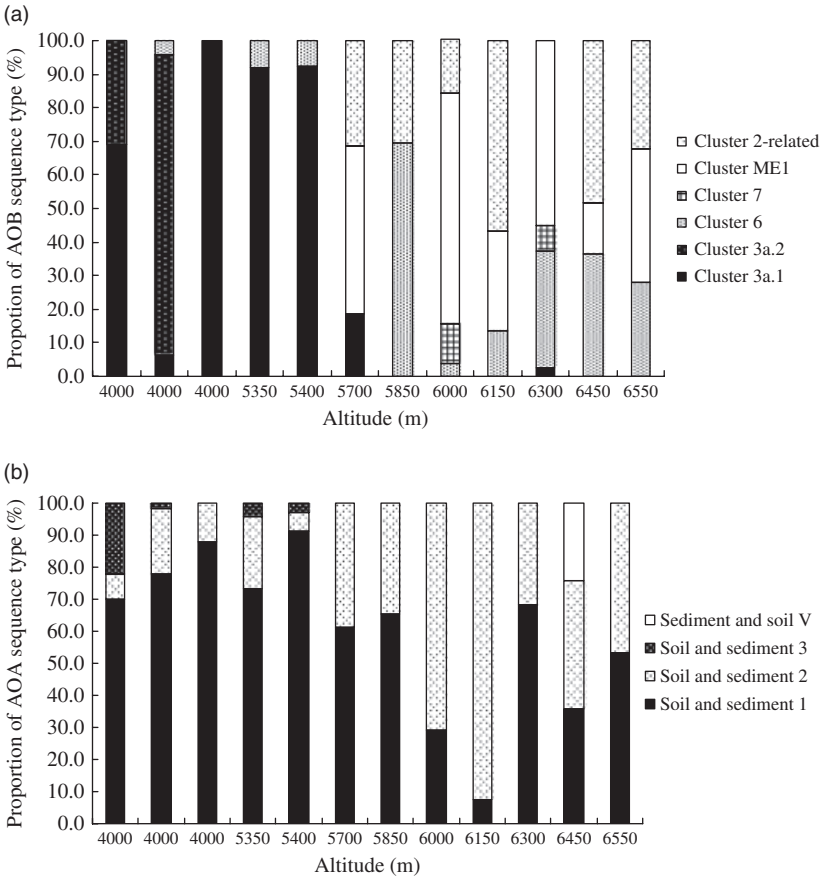

Fig. 5. Proportions of bacterial *amo*A (a) and archaeal *amo*A (b) gene clone types representing various clusters in soils from Mount Everest, on the Tibetan Plateau.

cluster 3a, whereas two newly designated *Nitrosospira* cluster ME and cluster 2-related sequences and *Nitrosomonas* cluster 6 were prevalent in the higher altitude soils. The permanent snow line in the northern slope of Mount Everest is at 5800–6000 m a.s.l. Soils above or near this altitude are permafrost, representing a harsher habitat with altitude-dependent physiochemical gradients including frozen temperature, stronger radiation, lower oxygen concentration and lower nutrients, which may determine the abundance and community structure of AOA/AOB in elevated soils.

Among these altitude-dependent factors, temperature has been clearly demonstrated to have a huge influence on the community structure of AOB and caused apparent population shifts within the different *Nitrosospira* clusters (Avrahami *et al*., 2003). Decreasing density of cluster 3a bands in denaturing gradient gel electrophoresis in response to a decrease in temperature, and a cluster 3a-dominated community at a high temperature were observed in previous studies (Avrhami & Conrad, 2003; Avrahami *et al*., 2003). This is consistent with the predominance of cluster 3a in lower altitude soils and lack of detection of this group in most high-altitude soils, suggesting a response to temperature. In contrast, *Nitrosospira* cluster ME and cluster 2-related sequences were most related to clusters 1, 2 and 4 and were present exclusively in higher altitude soils at high proportions. Clusters 1, 2 and 4 have so far only been found in cold temperate soils (Avrahami & Conrad, 2005). In soil microcosms, cluster 1 was found to be prevalent at a low temperature (4–10 °C), but disappeared completely at a high temperature (Avrahami *et al*., 2003). High proportions of *Nitrosomonas* cluster 6 were also observed in high-altitude soils. Studies based on the pure culture *Nitrosomonas cryotolerans* showed that they can grow at temperatures as low as –5 °C (Jones *et al*., 1988). Considering all these aspects, sequences belonging to cluster ME, cluster 2-related and *Nitrosomonas* cluster 6 may be representatives of a cold-tolerant AOB population and may be responsible for the observed higher abundance of AOB over AOA in these soils.

All AOA sequences fell within the soil and sediment clusters, suggesting that AOA communities in these alpine soils are distinct from those in aquatic environments. The proportions of the major cluster of AOA within clone libraries changed between lower and higher altitude soils, but no distinct horizon/site/treatment-specific clusters were observed as in the continuous soil profile (Hansel *et al*., 2008), in estuary (Mosier & Francis, 2008) and agricultural soil (Nicol *et al*., 2008; Di *et al*., 2009), where pH is acidic or near neutral. Pronounced AOA community changes in

response to long-term fertilization treatments were observed in acidic soil (He *et al.*, 2007), but not in alkaline soils with pH ranging from 8.3 to 8.7 (Shen *et al.*, 2008). Considering the high soil pH values (8.6–9.1) in this study, together with the above studies, no remarkable shift in the AOA community between lower altitude and high-altitude soils may be attributed to the high pH in the Tibetan Plateau.

In summary, distinct changes in the abundance and community composition of AOB were observed at altitudes near or above the permanent snow line, suggesting that altitude-dependent factors, in particular temperature, have a profound influence on the abundance and community of AOB. AOA abundance decreased significantly and was negatively correlated with altitude in this study. Bacterial *amo*A abundance was greater than archaeal *amo*A abundance in higher altitude soils, contrary to observations in many systems. This distinct change in the relative abundance of bacterial and archaeal ammonia oxidizers, and in the bacterial ammonia oxidizer communities in response to elevated altitude also suggests that these groups are sufficiently active to survive in these alpine areas. More recent studies have demonstrated that microbial metabolism proceeds at soil temperatures as low as –20 °C (Christner, 2002), and that CO_2 production, CH_4 uptake and N cycling occur in subnival soil (Sommerfeld *et al.*, 1993; Clein & Schimel, 1995; Brooks *et al.*, 1996; Lipson *et al.*, 1999), which supports this hypothesis. The ecological significance of the shifts in the relative abundance of AOA and AOB in these soils requires further study.

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Authors' contribution

L.-M.Z. and M.W. contributed equally to this work.

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